

Review Article

Detection of SARS-CoV-2 through pool testing for COVID-19: an integrative review

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Abstract

Introduction: The pool testing technique optimizes the number of tests performed and reduces the delivery time of results, which is an interesting strategy for the health crisis caused by the COVID-19 pandemic. This integrative review investigated studies in which pool testing was carried out for epidemiological or screening purposes to analyze its clinical or cost effectiveness and assessed the applicability of this method in high-, middle-, and low-income countries. **Methods:** This integrative review used primary studies published in the MEDLINE, EMBASE, *Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS)*, and Cochrane Library databases. **Results:** A total of 435 studies were identified: 35.3% were carried out in Asia, 29.4% in Europe, 29.4% in North America, and 5.9% in Oceania. **Conclusions:** This review suggests that pool testing in the general population may be a useful surveillance strategy to detect new variants of SARS-CoV-2 and to evaluate the period of immunogenicity and global immunity from vaccines.

Keywords: COVID-19. SARS-CoV-2. Pool testing. Diagnosis. Pandemic.

INTRODUCTION

As of the beginning of 2021, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been responsible for more than 3.5 million deaths and 165 million cases worldwide. These numbers placed the disease caused by SARS-CoV-2, coronavirus disease 2019 (COVID-19), as the most critical public health crisis in the last 100 years¹. Approximately 80% of infections are mild or asymptomatic cases, although these individuals are still contagious and contribute to viral transmission². When the

disease occurs without severe symptoms, people are less likely to recognize it and are therefore less likely to seek medical help, thus impairing prompt diagnosis, contact screening, and disease control. Symptomatic infections, as well as a substantial number of mild or asymptomatic infections, may go undetected in some countries. Moreover, confirmed case counts are biased owing to incomplete testing and low test sensitivity. Accurate estimates of the burden of SARS-CoV-2 infections are critical for understanding the course of the pandemic and informing the public health response³.

To understand the real epidemiological profile, incidence rate, prevalence, virulence, and lethality of the virus, it is necessary to test as many people as possible⁴. Cases are classified as asymptomatic⁵, symptomatic respiratory, flu-like syndrome, or severe acute respiratory syndrome⁶, based on clinical assessments complemented by laboratory tests.

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Tests to detect viral antigens, such as immunochromatography and enzyme-linked immunosorbent assays (ELISAs), are currently used to detect infected individuals. Nevertheless, real-time reverse-transcription polymerase chain reaction (RT-PCR) to detect viral ribonucleic acid (RNA) continues to be the most widely used diagnostic test and is the gold standard for confirming COVID-19. Tests to detect viral antigens or RNA rely on nasopharyngeal swabs or, in some cases, on bronchiolar secretions. These are usually collected in viral transport media and immediately forwarded to a diagnostic laboratory⁷.

Considering the importance of increasing the number of tests to detect SARS-CoV-2 in the population, a suitable alternative is to perform RT-PCR, which uses the pool testing strategy developed by Dorfman^{8,9}. Pool testing is a method of grouping several samples to be analyzed together. A second test on individual samples must be performed if a positive sample is present in the pool. If no positive result is detected in the pool, all samples are considered non-detectable^{10,11}. The number of samples per pool can vary according to the prevalence of infection (**Supplementary Table 1**)¹²⁻¹⁴.

Pool testing is currently a suitable strategy for coping with the COVID-19 pandemic. More than ever, it is necessary to expand access to COVID-19 diagnostic tests and to identify presymptomatic and asymptomatic individuals who contribute silently to the fast dissemination and maintenance of the pandemic¹⁵. Increased testing has also become essential as a tool to reduce the rapid dissemination of the virus and an increase in mutant variants, which are a growing threat worldwide¹⁶. This method can reduce the costs for health systems and increase testing scalability. It is known that the countries that were best able to control the pandemic were those that carried out mass tests in their populations. Therefore, pool testing has become an alternative method for mass testing. Pooling samples enables a rapid increase in screening capacity, which is essential to inform public health actions and to control the spread of COVID-19¹⁷.

Given the current importance of this topic, this study sought to review research that applied pool testing as a diagnostic method for SARS-CoV-2 in order to investigate the scenarios in which these investigations were carried out (epidemiological or screening, clinical analysis or cost-effectiveness analysis) and the applicability of this method in high-, middle-, and low-income countries.

METHODS

Search databases

This integrative review is based on primary studies published between March 1, 2020 and January 24, 2021 in the Medical Literature Analysis and Retrieval System Online (MEDLINE), *Excerpta Medica* database (EMBASE), *Literatura Latino-Americana e do Caribe em Ciências da Saúde* (LILACS), and Cochrane Library databases, as well as a search conducted for gray-literature articles through a manual search for references of selected articles or through Google Scholar (active search for local articles in references of found studies, sites, pre-prints, or conference annals). The search was not limited by the language of publication or by studies from high-, middle-, or low-income countries. To identify the references, a sensitive search was conducted.

This study included studies whose outcome variables were determined by the diagnoses reached using the pooling test. Variables of interest were the pooling test, comparisons between the pooling test and RT-PCR, and positivity profiles measured by direct methods. Observational (cross-sectional, case-control, cohort, and case studies), interventional (randomized controlled trials), or cost-effectiveness studies were included. Systematic reviews, meta-analyses, narrative reviews, and integrative reviews were excluded.

Screening criteria

Titles and abstracts of studies retrieved using the search strategy and those from additional sources were screened separately by two authors to identify studies that potentially met the inclusion criteria outlined above. The full texts of these potentially eligible studies were retrieved and independently assessed for eligibility by two review authors. Disagreements between reviewers on the eligibility of the studies were resolved through discussion with a third review author.

Data extraction

Two authors evaluated articles retrieved independently during eligibility searches using Mendeley Reference Manager (Mendeley, London, United Kingdom). Data extraction was performed using a standardized and pre-tested form for Microsoft Excel (Microsoft, Redmond, WA) to build an evidence-tuning worksheet.

The variables extracted from the articles were authors' names, affiliations, journals, countries of the studies, population, number of pools performed, number of samples per pool, number of detectable pools, percentage of detectable pools, percentage of individual detection within the detectable pools, type of information about the study/article, study category, and disclosure.

RESULTS

This study identified 425 articles from databases and 10 in the gray literature by a manual search for references of selected articles and through Google Scholar. Of the studies related to searching indexed databases, 367 studies were excluded by reading the titles and abstracts, leaving 58 studies for a full evaluation. During the evaluation of the texts, 12 studies were excluded because they did not apply pool testing to the studied population, 7 were excluded as pre-prints, and 22 were excluded for expressing an opinion on the subject without presenting numerical data. Thus, 17 studies were part of our sample, with 9 articles derived from the databases and 10 studies obtained from the gray literature. An overview of the selection process is presented in **Figure 1**.

Characteristics of studies

Table 1 summarizes the characteristics of the 17 individual studies published in 2020 (94.1%) and 2021 (5.9%) included in this integrative review. Of the studies included, 35.3% (n=6) were carried out in Asia, 29.4% (n=5) in Europe, 29.4% (n=5) in North America, and 5.9% (n=1) in Oceania (**Table 1**).

The articles were published in journals with impact factors ranging from 1.705 (24) to 21.770 (26), with 70.6% (n=12)

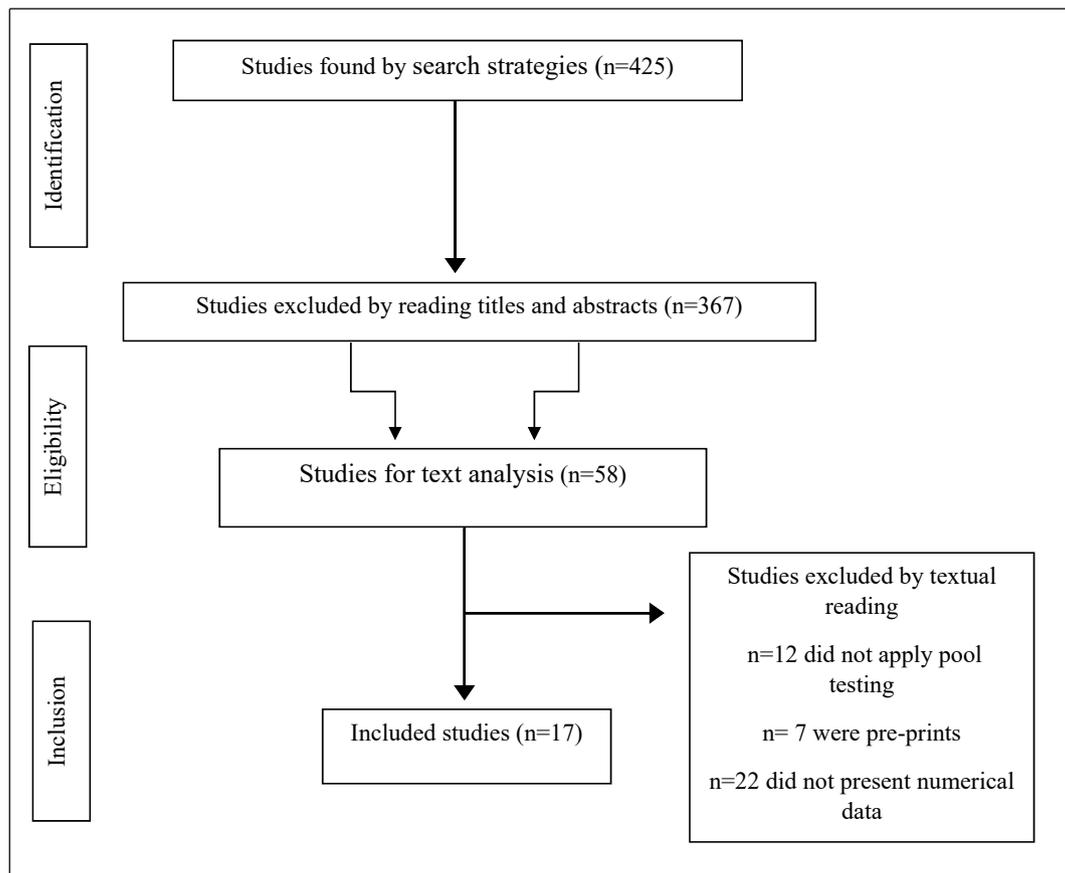


FIGURE 1: Study eligibility flowchart.

presented in journals with an impact factor greater than 2.000. In terms of study format, 41.2% (n=7) of the studies were original articles, 23.5% (n=4) were brief reports, 23.5% (n=4) were letters, 5.9% (n=1) were opinions, and 5.9% (n=1) were special articles. Pool testing was evaluated using cross-sectional studies (diagnostic accuracy) in 82.3% (n=14) of studies, as a case-control study in 11.8% (n=2) of studies, and as a case report in 5.9% (n=1) of studies. Regarding the institutions in which the studies were conducted, 76.4% (n=13) were carried out in universities, 17.4% (n=3) in research institutes, and 5.9% (n=1) in hospitals (**Table 1**).

Regarding the evaluated population, 29.4% (n=5) of studies evaluated samples taken from the general population, 23.5% (n=4) from health professionals, 23.5% (n=4) from asymptomatic patients, 11.8% (n=2) from patients with mild symptoms, and 11.8% (n=2) from hospitalized patients (**Table 1**). The 17 studies in this review were published between 2020 and 2021 in heterogeneous populations in 8 countries. The number of individual samples per pool ranged from 2 to 50 (number of samples per pool) with between 1 and 7,175 pool tests. The total number of individual samples in this review was 217,348, with between 10 and 59,476 samples per study. The positive pools and positive samples showed varied results, as shown in **Table 2**.

DISCUSSION

The objective of this review was to identify studies that applied the pool test as a diagnostic method for SARS-CoV-2

and to describe the research scenarios. We found studies from four different continents. Although RT-PCR is considered the gold standard for the diagnosis of COVID-19, it is an expensive test when performed individually, which can have an impact on a country's capacity to offer this test to the general population when following the epidemiology and/or natural history of the disease. Thus, due to the limited availability of test kits and their high cost, wider testing using the PCR method presents a challenge in coping with COVID-19³⁴. In this scenario, the pooled sample test (considered effective in screening for human immunodeficiency virus, chlamydia, malaria, and influenza) can be considered an important strategy to increase screening capacity and speed up tests for COVID-19³⁵.

Most of these studies were cross-sectional studies carried out by universities and aimed to establish ideal parameters for the combined group test for the detection of SARS-CoV-2, as well as to verify the feasibility of sample pooling as a strategy to increase test performance. Universities around the world play a crucial role in the development of new and more efficient technologies to assist in the diagnosis of COVID-19, either to develop strategies that optimize resources in this period of reagent shortage or to propose new, safer, and more cost-effective protocols, as shown by Schmidt et al³⁰. Of the studies evaluated in this review, only four were not conducted directly by universities, which highlights the importance of academic institutions for the development and optimization of techniques and the rational use of reagents.

TABLE 1: Characteristics of the studies included in the integrative review regarding COVID-19 diagnostics.

| Reference | Institute | Journal | Country | Population | Study category | Type of information |
|------------------------|---|---|-------------|--|------------------|----------------------------|
| Abdalhamid et al. (18) | University of Nebraska | AJCP | USA | People at risk for COVID-19 | Special article | Cross-sectional: Screening |
| Ben-Ami et al. (19) | The Hebrew University of Jerusalem | Clinical Microbiology and Infection | Israel | Asymptomatic | Original article | Cross-sectional: Screening |
| Chong et al. (20) | Victorian Infectious Diseases Reference Laboratory; University of Melbourne | Pathology | Australia | Hospital inpatients, healthcare workers | Brief report | Cross-sectional: Screening |
| Garg et al. (21) | Dr. Ram Manohar Lohia Institute of Medical Science | Journal of Medical Virology | India | General sample in areas with varying prevalence of population referred to COVID laboratory | Original article | Cross-sectional: Screening |
| Hogan et al. (22) | Stanford University School of Medicine | JAMA Network Open | USA | Patients with flu-like symptoms | Letter | Cross-sectional: Screening |
| Jung et al. (23) | University of Ulsan College of Medicine | JKMS | South Korea | Preoperative patients | Opinion | Cross-sectional: Screening |
| Lim et al. (24) | International Medical University | PLoS ONE | Malaysia | Contact tracing for COVID-19 | Original article | Cross-sectional: Inquiry |
| Lohse et al. (25) | Saarland University Medical Center | The Lancet Infectious Diseases | Italy | Asymptomatic | Letter | Cross-sectional: Screening |
| Mastrianni et al. (26) | Saratoga Hospital | Journal of Hospital Medicine | USA | Patients at low risk of SARS-CoV-2 infection | Brief report | Cross-sectional: Screening |
| Möckel et al. (27) | Universitätsmedizin Berlin | European Journal of Emergency Medicine | Germany | Health professionals | Letter | Case report |
| Mohanty et al. (28) | India Institute of Medical Sciences | Journal of Medical Virology | India | Asymptomatic or symptomatic people that had contact with infected patients | Original article | Cross-sectional: Screening |
| Petrucca et al. (29) | Sant'Andrea Hospital; University of Rome | Infection Control and Hospital Epidemiology | Italy | Health professionals | Letter | Cross-sectional: Screening |
| Schmidt et al. (30) | German Red Cross Blood Transfusion Service; Goethe-University | Transfusion | Germany | Symptomatic patients and asymptomatic employees | Original article | Cross-sectional: Screening |
| Denny et al. (31) | Duke University | Morbidity and Mortality Weekly Report | USA | Asymptomatic | Brief Report | Cross-sectional: Screening |
| Kim et al. (11) | Asan Medical Center; University of Ulsan College of Medicine | JKMS | South Korea | Health professionals | Brief Report | Cross-sectional: Screening |
| Schneitler et al. (32) | Saarland University; University of Cologne | Annals of Global Health | Germany | Police staff, hospital inpatients, and nursing home residents | Original article | Case-control |
| Wang et al. (33) | Stanford University | Emerging Infectious Diseases | USA | Symptomatic and asymptomatic inpatients and outpatients | Original article | Case-control |

NA : not applied.

TABLE 2: Main characteristics of the pools by studies included in this review.

| Reference | Country | Pools number | Total samples | Number of samples per pool | % Positive pools | % Positive sample | Sensitivity | Specificity |
|--------------------------|-------------|--------------|---------------|----------------------------|--------------------------------------|-------------------------------------|-------------|-------------|
| Abdalhamid et al. (2020) | USA | 12 | 60 | 5 | NS | 3.33% | 95% or 100% | 100% |
| Ben-Ami et al. (2020) | Israel | 3.322 | 26,576 | 8 | NS | 0.12% | NS | NS |
| Chong et al. (2020) | Australia | 7.175 | 29,700 | 4; 8 | 0.84% | 0.23% | 99% | 99% |
| Garg et al. (2020) | India | 3.452 | 19,560 | 5; 10 | 7.39% | 1.42% | 95% or 100% | 100% |
| Hogan et al. (2020) | USA | 292 | 2,888 | 9; 10 | 0.68% | 0.07% | NS | NS |
| Jung et al. (2020) | South Korea | NS | 37,127 | NS | NS | 0.30% | NS | NS |
| Lim et al. (2020) | Malaysia | NS | 2,732 | 5; 7; 10 | NS | 1.90% | NS | NS |
| Lohse et al. (2020) | Italy | 267 | 1,191 | 4; 30 | 4.24% | 1.93% | NS | NS |
| Mastrianni et al. (2020) | USA | 179 | 530 | 2-3 | 2.23% | 0.8% | 60% to 80% | 95% to 99% |
| Möckel et al. (2020) | Germany | 1 | 10 | 10 | 0% | 0% | NS | NS |
| Mohranty et al. (2020) | India | 1.807 | 7,228 | 4 | 8.3% | 3.47% | NS | NS |
| Petrucca et al. (2020) | Italy | 407 | 2,035 | 5 | 8.84% | 1.76% | 100% | 100% |
| Schmidt et al. (2020) | Germany | 3.210 | NS | 10; 20; 30; 40; 50 | NS | NS | NS | NS |
| Denny et al. (2020) | USA | NS | 59,476 | NS | n=158 | n=29 | NS | NS |
| Kim et al. (2020) | South Korea | NS | 609 | 5 | 0% | 0% | NS | NS |
| Schneitler et al. (2020) | Germany | 6.012 | 25,978 | 5; 10; 13 | 14.6% (random); 1.2% (questionnaire) | 3.5% (random); 0.1% (questionnaire) | NS | NS |
| Wang et al. (2021) | USA | 302 | 1,648 | 8; 4 | NS | 5.83% | 95% | NS |

NS: not specified.

Large proportions of the study populations were diagnosed as asymptomatic, thus illustrating the usefulness of this method as a screening tool for the disease, especially in more vulnerable populations. The management of SARS-CoV-2 transmissibility depends on the identification of infected individuals through validated laboratory tests with good sensitivity, specificity, and precision³⁶. The expansion of diagnostic capacity through increased testing and rapid results plays a key role in supporting decision-making in response to the COVID-19 pandemic. In addition, diagnosis is essential to conduct serological research that determines viral circulation in the community, to monitor disease trends over time, and to assist in control measures³⁷. The inequalities found in access to health in several countries around the world make it difficult for low- and middle-income populations

to perform diagnostic tests, especially the molecular test, which is currently considered the gold standard. Many individuals do not have adequate access to laboratory services in a timely manner, as they are in rural and remote areas or due to minimal financial resources^{37,38}.

The lack of diagnosis can generate a relatively low number of confirmed cases, with a great possibility of underestimating the data. One study has pointed out that low-income nations, like some African countries, have ineffective tests, underestimating incidence rates³⁹. Insufficient testing capacity in countries has become a barrier to case identification, quarantine, and contact tracing⁴⁰. Our study reveals that, at low prevalence locations, a pool size of around 30 appears to be effective³⁵, given the significant reduction in the

number of tests (depending on the size of the pool, stages, and pool design) and consequent lack of resources. It is important to mention that the risk of false negatives in the samples may increase owing to dilution of positive samples¹². Thus, low- and middle-income countries, where COVID-19 could exert extreme pressure on low-resource health systems, can be major beneficiaries of this type of diagnostic method⁴¹.

The prevalence of detection ranged from 0.84% to 14.6% and was higher in the general population and in health professionals. When discussing the screening of many people with no or few symptoms, establishing the correct pool for the given group tested according to COVID-19 prevalence can save up to 42% of reagents at a given prevalence of 10% in pools of 4 samples⁴². These values can be adjusted according to the region using mathematical models with the prevalence and number of samples. The pool method can offer an economical and effective approach to increase the virus testing capacity of medical laboratories without requiring more laboratory resources, such as laboratory workers, test reagents, and equipment⁴³. However, this method is not widely used, potentially due to a greater need for organization, systematization, and a multidisciplinary approach to create and adjust the pools. Pooling can help countries that are behind in testing by assessing specific groups, like essential workers and asymptomatic individuals⁴⁴, and provide opportunities to focus on other methods of control like contact tracing.

To prevent the spread of the virus, the detection of SARS-CoV-2 through pool testing has proven to be one of the key strategies to combat the pandemic. Along with the tracing, isolation, and regulation of contacts, detecting infected individuals is necessary to understand the evolution of the infection through statistics^{30,43}. Comprehensive testing of the population is important for understanding the curve and planning future strategies, which can be hampered due to the restriction of tests, reagents, and services⁴⁵. When planning laboratory operations, a well-coordinated strategy that uses a multidisciplinary team that understands the challenges mentioned above is more likely to succeed in such times of uncertainty⁴⁶.

Therefore, the pool testing method can drastically reduce costs when used with an appropriate organization, as it is more suitable in scenarios with a low prevalence of infection and in specific populations, such as asymptomatic patients, company workers, and civil servants⁴⁴. In addition, there are current perspectives published regarding the use of pool testing in the general population as a surveillance strategy to detect new variants of SARS-CoV-2 and to evaluate the period of immunogenicity and global immunity provided by vaccines^{16,47}.

CONCLUSION

Diagnostic pool testing of COVID-19 is being performed in several locations around the world, including developed countries. The shortage of studies in developing countries, where the pandemic may continue unrestrained, is noteworthy. We suggest that other sites around the world use pool testing as a screening or detection method of SARS-CoV-2 to contain the virus' transmissibility, given the sensitivity and specificity of the previously described method.

Through combining the experience developed in universities, the organizational tools of public health providers, and the technological apparatuses of laboratories, pool testing can be one of the many strategies applied in widespread testing.

AUTHORS' CONTRIBUTION

MSC, NSG, ABA contributed to the literature review, data interpretation, translation and writing the manuscript, critical manuscript review and preparation. MSC, NSG, ABA, LPVT, RBO, MSS, GOG, CRLA, ELM, FGF, SMRT, HIS, RHCT and UT contributed to the review, discussion and writing the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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